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Abstract
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ISOLATION, BIOLOGICAL ACTIVITIES AND SYNTHESIS OF THE NATURAL CASUARINES

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ABSTRACT: This chapter describes the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines. These are casuarine, casuarine-6-O-α-D-glucoside, 6-epi-casuarine (uniflorine A) and 3-epi-casuarine.

INTRODUCTION

Casuarine 1 [1], casuarine-6-O-α-glucoside 2 [2], 6-epi-casuarine 3 (uniflorine A) [3-5], and 3-epi-casuarine 4 [6] are members of the expanding group of polyhydroxylated 3-hydroxymethylpyrrolizidine natural products (Fig. (1)) [7]. This group also includes, australine [8], alexine [9] (7a-epi-australine), several other epi-australines (1-epi-australine, 3-epi-australine [10], 2,3-diepi-australine, 2,3,7-tri-epi-australine) [11], 1-epi-australine-2-O-α-glucoside and the more recently isolated hyacinthacine alkaloids of which nineteen novel compounds have been identified [12]. This group, along with the polyhydroxylated pyrrolidine, piperidine, indolizidine and nortropane alkaloids, have glycosidase inhibitory activities and thus have potential utility as
antiviral, anticancer, antidiabetic and antiobesity drugs [7]. Three structurally related synthetic compounds have been marketed as antidiabetic drugs to treat type-II diabetes based on their potent $\alpha$-glucosidase inhibitory activities while others have been identified as candidates for therapeutics to treat type-1 Gaucher disease [7]. These potentially useful biological activities, along with the stereochemical richness of these alkaloids, (uniflorine A and casuarine have six contiguous stereogenic carbons) has made these compounds attractive and important synthetic targets [13]. This chapter describes the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines.

![Structures of casuarines](image)

**Fig. (1).** Structures of casuarine 1, casuarine-6-$\alpha$-glucoside 2, 6-epi-casuarine 3 (uniflorine A) and 3-epi-casuarine 4

### Isolation of the Natural Casuarines

*Casuarina equisetifolia* L., or commonly called, Australian pine, Filao or beach she oak is a plant in the family *Casuarinaceae*, native to South
East Asia, islands of the western Pacific Ocean (including French Polynesia, New Caledonia, Vanuatu), Australia (Northern Territory, north and east Queensland, and northeastern New South Wales) and West Africa. It is an evergreen tree that grows to over 6-35 m in height [14]

The first pentahydroxylated pyrrolizidine alkaloid, with six contiguous stereogenic centres and functional groups on all of the eight carbon atoms, was isolated in 1994 from the bark of *Casuarina equisetifolia* L. [1]. This bark was prescribed as a remedy to treat breast cancer in Western Somoa [2]. Extracts of the wood, bark and leaves of this plant have also been claimed to be useful for the treatment of diarrhoea, dysentery and colic [1]. This alkaloid was named casuarine 1, (1R,2R,3R,6S,7R,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine), by Nash et al. [1]. This investigation started with a GC-MS analysis of the per-trimethylsilylated bark extract which revealed a pentahydroxylated pyrrolizidine alkaloid and its glycoside as the major alkaloid components. The 75% aqueous ethanol bark extract was purified by ion-exchange column chromatography with Amberlite CG 120 (NH₄⁺ form) which was eluted with 0.1 M NH₄OH to afford first the glycoside of casuarine 2 and then casuarine 1 itself (Fig. (I)). Both alkaloids were isolated in approximately the same amounts with latter in 0.013% yield based on the weight of the dried ethanol extract [1]. The absolute configuration of casuarine 1 was established by X-ray crystallographic analysis [1].

*Eugenia jambolana* is a plant in the family Myrtaceae, native to Bangladesh, India, Nepal, Pakistan and Indonesia. An evergreen tree it grows to 30 m in height. The extracts of the fruit pulp from *E.*
*jambolana* have been reported to have anti-diabetic properties, although this has been questioned in a more recent study [15]. In 1996, Wormald et al. [2] isolated casuarine 1 and its glucoside 2 from the leaves and the seeds of *Eugenia jambolana* using Amberlite CG 120 (NH₄⁺ form) ion exchange chromatography. From 630 g of air dried leaves they isolated 140 mg of casuarine 1 and 15 mg of the glucoside 2.

*Eugenia uniflora*, Surinam cherry, Brazilian cherry, or Cayenne cherry is a plant in the family Myrtaceae, native to tropical America and widely distributed in Paraguay, Uruguay, Argentina, and Brazil [3]. Decoctions of the leaves of this small tree are used as traditional medicines for a number of ailments, including use as an antidiabetic preparation. A number of studies have been made on the biological activities of the leaf extracts [16-18].

The water-soluble extracts of the leaves of *Eugenia uniflora* L. have been used as an antidiabetic agent in Paraguayan traditional medicine [3]. In 2000, Arisawa et al. [3] reported the isolation of uniflorine A and B from the leaves of this tree. The water-soluble extract was purified twice on Amberlite ion-exchange resins and then on silica gel and finally HPLC to give samples of uniflorine A, uniflorine B and (+)(3α, 4α, 5β)-1-methylpiperidine-3,4,5-triol in undisclosed amounts. The structures of the alkaloids uniflorine A and uniflorine B were deduced from NMR analysis to be that of the pentahydroxyindolizidine structures 3a and 1a, respectively (Fig. (5)).
In 2004, Pyne and Davis [19] synthesised the proposed structure of uniflorine A however the NMR spectral data for synthetic 3a, did not match with those reported for uniflorine A [3]. The structure of their synthetic 3a was unequivocally established by a single-crystal X-ray crystallographic study of its pentaacetate derivative. The Wollongong researchers therefore concluded that the structure originally assigned to uniflorine A was not correct [19]. The initial thoughts of several researchers were that uniflorine A was a diastereoisomer of 3a. In 2006, Dhavale et al. [20], in their paper of partial title, “Attempts To Find the Correct Structure of Uniflorine A.”, reported the second synthesis of compound 3a. Their sample of 3a had NMR spectral data identical to those of 3a that was earlier synthesised by Pyne et al. [20]. This paper also reported the synthesis of two diastereomers of 3a, 8a-epi-3a and 1,2,8a-tri-epi-3a. In 2005 Mariano et al. [21] reported the synthesis of 1-
epi-3a, while that of 1,2-di-epi-3a had been reported by Fleet et al. in 1996 [22], before uniflorine A was even isolated, and later by Mariano et al. [21] and by Pyne et al. in 2008 [4]. In 2008 Pyne et al. reported the synthesis of 2-epi-3a (Fig. (6)) [4]. Despite these synthetic chemistry efforts these 1,2,6,7,8-pentahydroxyindolizidine molecules also had NMR spectral data significantly different to those of uniflorine A.

![Proposed structure of Uniflorine A](image)

**1,2-di-epi-3a:**
Synthesis: 
Fleet, 1996
Mariano, 2005, Pyne, 2008

![Proposed structure of Uniflorine A](image)

**1-epi-3a:**
Synthesis: 
Mariano, 2005

![Proposed structure of Uniflorine A](image)

**2-epi-3a:**
Synthesis: 
Pyne, 2008

![Proposed structure of Uniflorine A](image)

**8a-epi-3a:**
Synthesis: 
Dhavale, 2006

![Proposed structure of Uniflorine A](image)

**1,2,8a-tripepi-3a**
Synthesis: 
Dhavale, 2006

Fig. (6). Synthesis of diastereomers of structure 3a.

From a re-examination of the original NMR data Pyne, Davis and Ritthiwigrom reassigned uniflorine B as the known pyrrolizidine alkaloid casuarine 1, while the structure of (-)-uniflorine A was suggested to be that of 6-epi-casuarine 3 (Fig. (5)) [4]. The structure of uniflorine A was unequivocally established to be that of 6-epi-casuarine 3 by its total synthesis (see synthesis section) [5, 23, 24].
*Myrtus communis* L., commonly known as Myrtle or True Myrtle, belongs to the family Myrtaceae. It originates from the Mediterranean, North African and Western Asia regions. Casuarine 1 and 3-*epi*-casuarine 4 were isolated *M. communis* L. growing in the grounds of the Institute of Grassland and Environmental Research in Aberystwyth, UK. The isolation was conducted using ion exchange chromatography. Casuarine 1 was the major alkaloid present, which eluted first with water from the anion exchange resin Dowex 1 (OH form) followed by 3-*epi*-casuarine 4 ((1R,2R,3S,6S,7R,7aR)-3-(hydroxymethyl)-1,2,6,7-tetrahydroxypyrrolizidine (Fig. 1)) No other epimer of 1 was isolated. Casuarine 1 and 3-*epi*-casuarine 4 were crystallized from warm 95% aqueous ethanol by layering with acetone. The absolute configuration of 3-*epi*-casuarine 4 was established by X-ray crystallographic analysis [6].

**Glycosidase Inhibitory Activities of the Natural Casuarines**

The inhibitory activities of casuarine 1 and casuarine-6-*O*-α-glucoside 2 against a panel of 14 glycosidases were examined. Casuarine 2 was a much more potent inhibitor of α-D-glucosidases (for example, rice α-D-glucosidase (IC\(_{50}\) 1.2 μM) and rat intestinal maltase (IC\(_{50}\) 0.7 μM)) than casuarine-6-*O*-α-glucoside 2 (for example, rice α-D-glucosidase (IC\(_{50}\) 440 μM) and rat intestinal maltase (IC\(_{50}\) 260 μM)) [11]. In contrast, casuarine-α-glucoside 2 was a more active inhibitor of β-D-glucosidase from almond (IC\(_{50}\) 7.0 μM). Both compounds 1 and 2 were potent inhibitors of amyloglucosidase from *Aspergillus niger*, with IC\(_{50}\) values of 0.7 μM and 1.1 μM, respectively [11].
Casuarine 1 and casuarine-6-O-α-glucoside 2 were found to be inhibitors of the human N-terminal subunit of maltase-glucoamy lase (NtMGAM) and *Escherichia coli* trehalase (Tre37A). Casuarine 1 and casuarine-6-O-α-glucoside 2 had *Ki* values of 0.45 μM and 280 μM, respectively, against human NtMGAM and *Ki* values of 17 μM and 12 nM, respectively against Tre37A [25]. The high potency of casuarine-6-O-α-glucoside 2 against Tre37A is most significant. These studies confirmed an earlier study that showed casuarine 1 and casuarine-6-O-α-glucoside 2 were active inhibitors of trehalase from porcine kidney with IC₅₀ values of 12 μM and 0.34 μM, respectively [11].

There is current interest in inhibitors of these enzymes. Human maltase-glucoamylase is one enzyme involved in the digestion of starch to glucose. Inhibitors of this enzyme can be used to control the rate of glucose production and thus potentially aid in the treatment of type-II diabetes [26]. Trehalase is found mainly in the midgut of insects and converts trehalose, the major sugar in the blood of insects, to glucose which is vital for insect flight. Thus inhibitors of this enzyme may have potential as insecticides [26, 27]. X-ray crystal structures of the complexes of casuarine 1 with human NtMGAM and casuarine-O-α-glucoside 2 with Tre37A were determined and revealed similarities in the catalytic sites of these unrelated enzymes [25]. Computer-aided docking studies of casuarine 1 into the active site of NtMGAM were consistent with the X-ray crystal structure and both studies indicated that all 5 hydroxyl groups of casuarine 1 are involved in H-bonding to amino acid residues in the active site and the protonated nitrogen atom of casuarine 1 forms a salt bridge with Asp443 [28].
In another study, casuarine-6-O-α-glucoside 2 was found to be an nM inhibitor of trehaloses from midge larvae (*Chironomus riparius*), mammalian pig kidney and *E. coli*. Significantly, casuarine-6-O-α-glucoside 2 and two of its analogues were 10 or more times more potent on the insect trehalase than the other two enzymes indicating their potential as selective insecticides [29]. Other studies showed that casuarine 1 inhibited a membrane-bound trehalase from midge larvae (*C. riparius*) with an IC₅₀ of 250 nM [30].

Uniflorine A and B were found to be inhibitors of the α-glucosidases, rat intestinal maltase (IC₅₀ values of 12 and 4.0 μM, respectively) and sucrase (IC₅₀ values 3.1 and 1.8 μM, respectively) [3]. The biological activity of the leaf extracts may be a result of the glycosidase inhibition activities of the natural product components, including the alkaloids uniflorine A and B [3]. The structures of these two alkaloids were later revised to be that of 6-epi-casuarine 3 and casuarine 1, respectively [5, 23, 24]. In 2010, the results of the glycosidase inhibitory testing of 6-epi-casuarine 3 at 143 μg/mL showed 94-97% inhibition against the α-D-glucosidases of *Saccharomyces cerevisiae* and *Bacillus sterothermophilus* and against the amylglucosidase of *Aspergillus niger*. The IC₅₀ values were only determined for the two aforementioned α-D-glucosidases and were found to be modest at 34 and 28 μM, respectively [31]. In the same assays, casuarine 1 had IC₅₀ values of 139 μM and 5.6 μM, respectively [32].

In contrast to casuarine 1, 3-epi-casuarine 4 showed weak activity against three α-D-glucosidases (from yeast, rice and *Bacillus*) and was more active against β-D-glucosidase from almond (IC₅₀ ca. 700
μM). In the same assay, casuarine 1 showed 0% inhibition of this latter enzyme at a concentration of 700 μM, while castanospermine (“the bench mark for β-D-glucosidase inhibition”) had an IC₅₀ of 20 μM [6].

**Synthesis of the Natural Casuarines**

*Synthesis of casuarine 1*

The first synthesis of casuarine 1 was achieved by Denmark *et al.* [33a,b] in four additional steps from a key tandem [4+2]/[3+2] nitroalkene cycloaddition reaction in 20% overall yield. The synthesis commenced with the preparation of the enantiomerically enriched (98% ee) vinyl ether 7 (Scheme 1). The chiral, alkoxy aldehyde 5 [33c] was converted to the silyl enol ether 6 in 99% yield as a 10:1 (Z/E) mixture. O-Benzoylation of 6 with benzoyl fluoride (BzF) and a catalytic amount of tetrabutylammonium fluoride (TBAF) (2 mol%) provided 7 as a mixture of Z and E vinyl ethers, which were separated by silica gel chromatography in yields of 81% and 6%, respectively (Scheme 1).

![Scheme 1. Synthesis of the chiral vinyl ether 7. Reagents and conditions: (a) TMSCl, Et₃N, CH₃CN, 81 °C, 99%; (b) BzF, TBAF, THF, 0 °C, 2 h, (81% Z: 6% E).](image)

The chiral nitronate 9, the 1,3-dipolar component of the [3+2] cycloaddition, was prepared from an *endo*-diastereoselective [4+2]-
cycloaddition reaction of the nitroalkene 8 and the chiral vinyl ether 7 in the presence of 2.5 equiv of SnCl₄ in toluene at -78 °C (Scheme 2). The nitronate 9 was not stable and was immediately treated with the 1,1,2-trimethylpropylsilyl (TDS) protected β-phenyldimethylsilyl enone 10 to give a 45:7:3:2:1:1 mixture of six isomeric cycloadducts in 76% yield. Purification by HPLC afforded the desired nitroso acetal 11 in 55% overall yield. Reduction of the ketone group of 11 with L-selectride at -78 °C led to a 10:1 mixture of epimeric alcohols 12 in 87% yield. Mesylation of the secondary alcohol 12 gave the mesylate 13 in 97% yield. This mesylate was converted to the pyrrolizidine 14 in 64% yield via hydrogenolysis over Raney nickel and then hydrolysis of both benzoate esters under basic conditions. The final step was transformation of the C-1 silyl group to the final hydroxyl substituent (Tamao-Fleming reaction) by dearylation of the phenyldimethylsilyl group with mercuric trifluoroacetate in trifluoroacetic acid, followed by oxidation with peracetic acid to afford pure casuarine 1 in 84% yield after ion-exchange column chromatography. Since aldehyde 5 can be prepared from diphenylacetonitrile in six overall steps [33c] the total steps in this synthesis of casuarine 1 are 14 (or 13 if you count the one-pot reaction going from 8 to 11).
Scheme 2. Total synthesis of casuarine 1 by Denmark et al. [33a,b]. Reagents and conditions: (a) Z-7, SnCl₄, toluene, -78 °C; (b) 10, CHCl₃; (c) L-Selectride, THF, -78 °C, 87% (10:1); (d) Ms₂O, py, 1 h, 97%.

Scheme 2. (Continued) Total synthesis of casuarine 1 by Denmark et al. [33a,b]. Reagents and conditions: (e) i: Raney Nickel, MeOH, 260 psi H₂; ii: K₂CO₃, MeOH, rt, 64%; (f) Hg(OTFA)₂, TFA, HOAc, AcO₂H, 84%.
A synthesis of casuarine 1 and its 6,7-diepimer, in a stereocontrolled manner, was reported by Izquierdo et al. [34a]. The synthesis of casuarine 1 began with N-Cbz protection of the DMDP derivative 16 [34b] that gave the Cbz compound 17 in 93% yield (Scheme 3). Primary alcohol oxidation and then a Wittig reaction of 18 gave the pyrrolidinic propenoate 19 (in 2 steps). Dihydroxylation of 19 using osmium tetraoxide and NMO in the presence of O-(4-chlorobenzoyl)hydroquinine (DHQ-CLB) as a chiral ligand gave a mixture of 20 and 21 in yields of 58% and 27%, respectively. The configuration of both diol products could not be determined at this stage. After two more steps, an NOE experiment confirmed that 20 was the desired intermediate to make casuarine 1. N-deprotection of 20 under hydrogenolysis reaction conditions provided pyrrolidine 22 which was subsequently transformed to 23 by heating a methanol solution at reflux in the presence of a catalytic amount of NaOMe.
Scheme 3. Total synthesis of casuarine 1 by Izquierdo et al. [34a]. Reagents and conditions: (a) CbzCl, Me₂CO, K₂CO₃, rt, 93%; (b) TPAP, NMO, 4Å MS, CH₂Cl₂; (c) Ph₃P=CHCO₂Me, CH₂Cl₂, rt, 85% (from 17); (d) OsO₄, NMO, DHQ-CLB, acetone/H₂O, rt, 2 d. (20:21 = 58%:27%); (e) H₂, 10% Pd-C, MeOH; (f) cat. MeONa, MeOH, rt, 85%; (g) BH₃·SMe₂, THF, then MeOH, Δ, 89%; (h) n-Bu₄N⁺·3H₂O, THF, rt, 95%; (i) i: H₂, 10% Pd-C, MeOH, then Amberlite IRA-400 (OH⁻ form), ii: Ac₂O, py, DMAP, 41%; (j) cat. NaOMe, MeOH, rt, 93%.
Reduction of the lactam carbonyl group of 23 using BH₃·SMe₂ complex gave 24 in 89% yield. O-TBDPS deprotection and then debenzylation of 24 gave 25 in 95% yield. Hydrogenolysis then gave an impure sample of 1. This sample was further purified by peracetylation which gave 26 in 41% yield. Base catalysed deacetylation of 26 afforded casuarine 1 in 93% yield. This synthesis was achieved in 8 steps from the DMDP derivative 16 in 13 % overall yield. DMDP 16 was prepared in 5 steps from a compound derived from D-fructose [34b], thus the total number of synthetic steps using this route is more than 13.

In 2006, Fleet et al. [6] published the synthesis of casuarine 1 from D-gluconolactone 27 (Scheme 4). D-Gluconolactone 27 was reacted with 2,2-dimethoxypropane and the open chain diacetonide, was subsequently esterified with trifluoromethanesulfonic anhydride to afford the triflate 28 in 72% yield. The triflate group of 28 was displaced with sodium azide in DMF to give the azide 29 in 97% yield. The unsaturated ester 30 was obtained from reduction of the azidoester 29 with DIBAL, followed by treatment of the resulting aldehyde with the Wittig reagent, Ph₃P=CHCO₂Me, in 75% yield over the two steps. The unsaturated ester 30 had an E:Z ratio of 10:1. After isolation of the pure E isomer of compound 30 it was converted to a mixture (1:4) of the diols 31 and 32, using an OsO₄ catalysed dihydroxylation reaction, in 72% yield. Hydrogenation of this mixture gave a mixture of amines which cyclized to the pyrrolizidine framework upon heating in toluene. Finally, after treatment of the reaction mixture with TBSCI, the lactam 33 was separated in 70% yield over the 3 steps. The terminal acetonide of 33 was removed by acid hydrolysis to afford the diol 34 in 69% yield. Selective protection of the primary hydroxyl group of diol 34 with
TBSCl gave the secondary alcohol 35 in 81% yield. The remaining secondary hydroxyl group of 35 required inversion of its configuration. This was achieved by treatment with triflic anhydride to afford the unstable triflate 36 which was reacted with caesium trifluoroacetate. Base hydrolysis of the resulting trifluoroacetate gave the inverted alcohol 37 in 20% yield over the 3 steps. The desired lactam mesylate 38 was obtained in 90% yield by treating 37 with methanesulfonyl chloride. Reduction of the lactam carbonyl group of 38 with BH$_3$·THF gave the amine 39 (57% yield). Finally pure casuarine 1 was obtained after 2 more steps, O-silyl group hydrolysis with TFA and then cyclization by treatment with sodium acetate (91% yield over the two steps). Overall the total number of synthetic steps was 13 starting from D-gluconolactone 27.

Scheme 4. Total synthesis of casuarine 1 by Fleet et al. [6]. Reagents and conditions: (a) Me$_2$C(OMe)$_2$, p-TsOH, MeOH; then (CF$_3$SO$_2$)$_2$O, py, CH$_2$Cl$_2$, 72%; (b) NaN$_3$, DMF, 97%; (c) t-Bu$_2$AlH, -78 °C; then Ph$_3$P=CHCO$_2$Me, toluene (75% over two steps); (d) cat. OsO$_4$, NMO, t-BuOH/H$_2$O, 72%.
Scheme 4. (Continued) Total synthesis of casuarine 1 by Fleet et al. [6]. Reagents and conditions: (e) H₂, Pd/C, THF; then toluene, Δ; then t-BuMe₂SiCl, imidazole, THF (70% over three steps); (f) 60% HOAc, H₂O/MeOH, 69%; (g) t-BuMe₂SiCl, py, 81%; (h) (CF₃SO₂)₂O, py, CH₂Cl₂; (i) CF₃CO₂Cs, 2-butanone; then K₂CO₃, MeOH (20% from 35); (j) CH₃SO₂Cl, Et₃N, CH₂Cl₂, 90%; (k) BH₃·THF, THF, 57%; (l) 90% CF₃CO₂H, H₂O; (m) NaOAc, H₂O (91% over two steps).

In 2009, Goti et al. [25a] published the synthesis of casuarine 1 and the first total synthesis of its 6-O-α-glucoside 2. Their key steps were a 1,3-dipolar cycloaddition reaction, a Tamao-Fleming reaction and a Mitsunobu reaction. Their total synthesis (Scheme 5) began with a stereoselective cycloaddition reaction of the nitrone 41 with the alkene 42 in CH₂Cl₂ to give the isoxazolidine 43. N-O bond cleavage of 43 with
Zn/HOAc then attack of the amine on to the ester carbonyl group resulted in the lactam 44. This compound was converted to 45 using the Tamao-Fleming reaction similar to that employed by Denmark (Scheme 2) [33a,b]. Reduction of lactam 45 with LiAlH₄ gave 46 in 76% yield, which was debenzylated under standard hydrogenolysis reaction conditions to give pure casuarine 1 in five steps and 44% overall yield from the nitrone 41. This latter compound is prepared in seven steps from L-xylose or D-arabinose [25b] making the total number of synthetic steps for the synthesis of casuarine 1 as 12 and the total overall yield 19%.

Scheme 5. Total synthesis of casuarine 1 by Goti et al. [25a]. Reagents and conditions: (a) CH₂Cl₂, rt, 36 h, 79%; (b) Zn, AcOH/H₂O, 60-65 °C, 5 h, 93%; (c) Hg(CF₃CO₂)₂, TFA, AcOH, AcOOH, CH₂Cl₂, 76%; (d) LiAlH₄, THF, reflux, 78%; (e) H₂, Pd/C, MeOH, HCl, 100%.
The total synthesis of casuarine by Ritthiwigrom and Pyne was completed in 13 steps and 8% overall yield (Schemes 6 and 7) [23]. The 1,2-anti aminoalcohol 47 was obtained from the boronic acid-Mannich reaction (Petasis reaction) of L-xylose, allylamine, and (E)-styrene boronic acid [19, 35] in 92% yield as a single diastereomer after purification by ion-exchange chromatography. The amino-tetraol 47 was converted to its N-Boc derivative 48 (80% yield) and then the terminal diol functionality of 48 was selectively protected as the acetonide derivative 49 under standard conditions. A ring-closing metathesis reaction of the diene 49 using Grubbs’ first-generation ruthenium catalyst 50 provided the 2,5-dihydropyrrole 51 in 97% yield (Scheme 6).

![Scheme 6. Synthesis of 51. [23] Reagents and conditions: (a) (E) PhCH=CHB(OH)2, allyl amine, EtOH, rt, 3 d; ion-exchange, 92%; (b) (Boc)2O, Et3N, MeOH, rt, 3 d, 80%; (c) DMP, PPTS, acetone, rt, 20 h, 64%; (d) Grubbs’ I 50, CH2Cl2, 50 °C, 18 h, 97%.

The synthesis of casuarine 1 from the chiral 2,5-dihydropyrrole 51 is shown in Scheme 7. To secure the 6α,7β-configuration of the target molecule the synthetic plan involved a regioselective ring-opening reaction of the epoxide 58 with an oxygen nucleophile (Scheme 7).
Scheme 7. Total synthesis of casuarine 1 from the precursor 51 by Ritthiwigrom and Pyne [23]. Reagents and conditions: (a) NaH, BnBr, n-Bu4NI, THF, 18 h, 92%; (b) HCl/MeOH, rt, 30 h, 76%; (c) TBSCI, DMAP, imidazole, THF, rt, 1 d, 81%; (d) FmocCl, THF, sat. Na 2CO3, 0 °C, 3 h, 94%; (e) CF3COCH3, oxone, NaHCO3, MeCN/H2O, 0 °C, 2 h, 81%; (f) MsCl, Et3N, CH2Cl2, N2, 0 °C, 3 h, 94%; (g) piperidine, MeCN, rt, 15 h, 96%; (h) NaHSO4, CH2Cl2, reflux, 2 d; water, rt, 1 h, 51%; (i) PdCl2, H2 (1 atm), MeOH, rt, 1.5 h; ion-exchange, 93%.

To obtain the key epoxide 58, the two unprotected secondary hydroxyl groups in 51 were protected as their O-benzyl ethers and the resulting dibenzyl ether 52 (92% yield) was treated under acidic conditions to effect hydrolysis of both the acetonide and N-Boc
protecting groups and to provide amino diol 53 in 76% yield. Regioselective O-silylation of 53 at the primary hydroxyl group gave the TBS ether 54 (81% yield) which was efficiently N-protected as its Fmoc derivative 55 in 94% yield. Epoxidation of the alkene moiety of 55 using 1,1,1-trifluoroacetone and oxone [36] provided the epoxide 56 in 81% yield as a single diastereomer. O-mesylation of the free secondary hydroxyl of 56 followed by treatment of the mesylate 57 (94% yield) with piperidine resulted in smooth N-Fmoc deprotection and then cyclization of the free cyclic secondary amine to give in 96% yield a 91:9 mixture of the desired pyrrolizidine 58 and the undesired indolizidine 58a, respectively. It was assumed that 58 arose from O-TBS migration under the basic conditions of the O-mesylation reaction. Fortunately, pure 58 could be obtained by further separation of the mixture by column chromatography. The structure of the epoxide 58 was confirmed by a single crystal X-ray analysis. Several attempts in our laboratory to ring-open the epoxide group of compounds related to 58 using aqueous acid conditions (for example, H2SO4/water) led to complex mixtures and low yields of diol products. However, when 58 was treated under the conditions reported by Saracoglu [37], using NaHSO4 as both the acid catalyst and the nucleophilic species in dichloromethane at reflux, followed by the addition of water to hydrolyze the intermediate sulfate, then the desired diol 59 was obtained as an 86:14 mixture of regioisomers. Purification of this mixture by column chromatography gave a 92:8 mixture of the diastereomeric diols 59 and 6,7-di-epi-59, respectively in 51% yield. The regiochemistry of this ring-opening reaction was consistent with that reported on related epoxy-pyrrolizidines [38] and was expected from stereoelectronic
considerations as shown in Scheme 8. For trans-1,2-diaxial ring opening of epoxide 58 by HSO₄⁻, the two reactive conformations, A and B are possible. Attack on conformation A at C-7 is inhibited by 1,3-diaxial interactions between the nucleophile (HSO₄⁻) and the pseudo-axial protons H-1α and H-5α and thus addition to conformation B at C-6 predominates resulting in 59 as the major regioisomeric product. Hydrogenolysis of 59 over PdCl₂/H₂ gave casuarine 1, in 93% yield after purification by ion-exchange chromatography. The diastereomeric purity of 1 was 95:5 from ¹H NMR spectroscopic analysis.

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\text{Scheme 8. Ring-opening reactions of epoxide 58 via conformations A and B [23].}
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Thus there have been five total syntheses of casuarine 1, with that of Goti being the shortest and most efficient with a total of 12 synthetic steps from D-arabinose in an overall yield of 19%. The other four syntheses involve 13 or more steps, and unlike that of Goti, include the
separation of unwanted regioisomers, diastereomers or \((E)\) and \((Z)\) isomers. Further the Goti synthesis allows for the ready preparation of casuarine-6-\(O\)-\(\alpha\)-\(D\)-glucoside 2 (Scheme 9).

*Synthesis of casuarine-\(O\)-\(\alpha\)-glucoside* 2

In 2009, Goti et al. [25a] reported the synthesis of casuarine-6-\(O\)-\(\alpha\)-\(D\)-glucoside 2 in the same publication in which they reported the synthesis of casuarine 1. The synthesis of casuarine-6-\(O\)-\(\alpha\)-\(D\)-glucoside 2 started with the same precursor 44, followed by acetylation and the Tamao-Fleming reaction for the oxidation of the C-Si bond to afford the alcohol 60 (Scheme 9).
Scheme 9. Total synthesis of casuarine-6-O-α-D-glucoside 2 from the precursor 44 by Gotti et al. [25].
Reagents and conditions: (a) Ac₂O, pyridine, rt, 15 h, 100%; (b) Hg(CF₃CO₂)₂, TFA, AcOH, AcOOH, CHCl₃, 82%; (c) BnOC(=NH)CCl₃, CF₃SO₃H, Et₂O, rt, 3 h; (d) Ambersep 900 OH, MeOH, rt, 15 h, 75% (2 steps); (e) TMSOTf, Et₂O, -20 °C, 40 min, 72%; (f) LiBH₄, BH₃-THF, THF, 23°C, 3 d, 68%; (g) H₂, Pd/C, MeOH, HCl, 77%.

Then, protection of secondary alcohol of 60 followed by an acetyl group deprotection sequence gave 61 in 75% yield over the 2 steps. Compound 63 was prepared by a coupling reaction between the alcohol 61 and the trichloroacetimidate 62. The glucoside alkaloid 2 was obtained by reduction of the lactam carbonyl of 63 and then deprotection of 64 by hydrogenolysis over Pd/C.
**Synthesis of 6-epi-casuarine (uniflorine A) 3**

The structural reassignment of uniflorine A to that of 6-epi-casuarine 3 was unequivocally confirmed in 2008 by Ritthiwigrom and Pyne on the basis of the total synthesis of ent-6-epi-casuarine 3 [(+)-uniflorine A] from D-xylose in 11 synthetic steps [5]. The NMR spectral data of the synthetic compound matched almost perfectly with that of the natural product. The specific rotation of synthetic (+)-uniflorine A (ent-6-epi-casuarine 3) ([α]^{22}_D + 6.6 (c 0.35, H₂O) was essentially equal in magnitude and opposite in sign to that of the natural product (-)-uniflorine A, ([α]_D -4.4 (c 1.2, H₂O)). In 2010 these workers reported the total synthesis of the correct enantiomer of natural uniflorine A in 11 steps and 13% overall yield. This synthesis was the same as the previous one except that the starting material was D-xylose rather than L-xylose [23]. The synthesis of (-)-uniflorine A 3 from the chiral 2,5-dihydropyrrole 51 is shown in Scheme 10. This intermediate was readily prepared on a 4 g scale from L-xylose in 4 steps and in 46% overall yield. The 2,5-dihydropyrrole 51 underwent an osmium(VIII)-catalyzed syn-dihydroxylation (DH) reaction to furnish the tetrol 65 as a single diastereomer in 72% yield (Scheme 10). The stereochemical outcome of this DH reaction was expected due to the stereodirecting effect of the C-2 pyrrolidine substituent in 51 [4, 19, 39, 40]. The configuration of this diol was established by ROESY NMR studies on the final product 3. The tetrol 65 was readily converted to its per-O-benzyl protected derivative 66 in 96% yield using standard reaction conditions. [19] Treatment of 66 under acidic conditions (HCl/MeOH) resulted in N-Boc and acetonide hydrolysis and gave the amino diol 67 in 81% yield.
Regioselective $O$-silylation of $67$ with TBSCl/imidazole/DMAP gave the primary silyl ether $68$ in 85% yield. In the earlier synthesis of (+)-uniflorine A, compound $ent$-$68$ underwent cyclization under Mitsunobu reaction conditions using pyridine $[4, 41-43]$ as the solvent to give a mixture ($ca$ 4:1) of the desired pyrrolizidine $ent$-$69$ and an indolizidine product (structure not shown) in a combined yield of about 30% after purification of the crude reaction mixture by column chromatography. The undesired indolizidine product arose from first base catalyzed $O$-
TBS migration to the secondary hydroxyl group in $ent$-$68$ followed by Mitsunobu cyclization onto the primary carbon of the butyl side chain. It was found that by buffering the reaction mixture with Et$_3$N·HCl $[44]$ that the yield of $69$ could be dramatically improved to 76% with little or no formation of the undesired product. Acid hydrolysis of $69$ gave the primary alcohol $70$ in 90% yield which upon hydrogenolysis using PdCl$_2$/H$_2$ gave uniflorine A $3$ in 87% yield after ion-exchange chromatography in a total of 11 synthetic steps and 13% overall yield from L-xylose.
Scheme 10. Synthetic route for (-)-uniflorine A 3 by Ritthiwigrom and Pyne [23]. Reagents and conditions:
(a) K₂OsO₄·H₂O, NMO, acetone/H₂O, rt, 18 h, 72%; (b) NaH, BnBr, n-Bu₄NI, THF, 24 h, 96%; (c) HCl/MeOH, rt, 18 h, 81%; (d) TBSCI, DMAP, imidazole, CH₂Cl₂, rt, 48 h, 85%; (e) DIAD, Ph₃P, Et₃NHCl, py, rt, 3 d, 76%; (f) HCl/MeOH, rt, 18 h, 90%; (g) PdCl₂, H₂ (1 atm), MeOH, rt, 24 h; ion-exchange, 87%.

In 2009, Goti et al. [24] reported the total synthesis of (-)-uniflorine A 3 in 9 steps and 11% overall yield from 41 or 16 steps from L-xylose or D-arabinose [25b] (Scheme 11). Their syntheses started with the 1,3-dipolar cycloaddition reaction product 43 that they used earlier to prepare casuarine (Scheme 5). The lactam 71 was obtained from cleavage of the N-O bond in 43 with Zn/HOAc followed by attack of the resulting amine onto the ester carbonyl group to form a hydroxy lactam. This intermediate was then acetylated to give the lactam 71 in 93%.
yield. The Tamao-Fleming reaction (Hg(CF₃CO₂)₂, TFA, AcOH, AcOOH) was used to convert the silyl group in 71 to the hydroxyl group in lactam 72 with retention of configuration. Benzylation of this hydroxyl group with BnOC(=NH)CCl₃, and CF₃SO₂H in Et₂O, also resulted in deprotection of the acetyl group at C-6, and provided compound 73 in 75% yield. The pyrrolizidine 75 was obtained by inversion of the stereochemistry at the C-6 position of compound 73 by a Mitsunobu reaction with BzOH, PPh₃, DIAD in THF that gave 74 in 75% yield. This was then followed by reduction of the lactam carbonyl group and deprotection of the benzoylated group with LiAlH₄. Debenzylation of the pyrrolizidine 75 using standard hydrogenolysis conditions gave (-) uniflorine A 3 in 71% yield after purification by ion-exchange chromatography with Dowex 50WX8 resin. The ¹H NMR and ¹³C NMR spectroscopic data was identical with those from our previous synthesis [5]. This synthetic material 3 had a mp 117-180 °C and an [α]²¹D -6.9 (c 0.42, H₂O).
Scheme 11. Total synthesis (-)-uniflorine A 3 by Goti et al. [24]. Reagents and conditions: (a) CH₂Cl₂, rt, 36 h, 79%; (b) i: Zn, AcOH/H₂O, 60–65 °C, 5 h, 93%; ii: Ac₂O, py, rt, 15 h, 100%; (c) Hg(CF₃CO₂)₂, TFA, AcOH, AcOOH, CHCl₃, 82%; (d) i: BnOC(=NH)CCl₃, CF₃SO₃H, Et₂O, rt, 3 h; ii: Ambersep 900 OH, MeOH, rt, 15 h, 75% (2 steps); (e) BzOH, PPh₃, DIAD, THF, rt, 75%; (f) LiAlH₄, THF, reflux, 45%; (g) H₂, 10% Pd/C, MeOH, HCl, rt, then Dowex 50WX8, 6% NH₄OH, 71%.

Synthesis of 3-epi-casuarine 4

In 2006, Izquierdo et al. [45] published the synthesis of 3-epi-casuarine 4 in the same year that Fleet et al. [6] reported its isolation as a natural product and also its synthesis. The synthesis of 3-epi-casuarine 4 by
Izquierdo et al. [45] involved the same methodology that they used for the synthesis of casuarine 1 (Scheme 3) except using the pyrrolidine 76 as the starting material. N-Cbz protection of 76 gave the Cbz carbamate 77 in only 25% yield (Scheme 12). The primary alcohol of 77 was oxidized using TPAP and NMO to afford the aldehyde 78 which after a Wittig reaction gave the (E)-pyrrolidinic propenoate 79 (93% yield). A cis-DH reaction of 79 using osmium tetroxide and NMO in the presence of O-(4-chlorobenzoyl)hydroquinine (DHQ-CLB) as a chiral ligand gave the diols 80 (13% yield) and 81 (84% yield). The configuration of these diol products could not be determined at this stage. After two more synthetic steps an NOE experiment confirmed that 82 was the desired intermediate to prepare 3-epi-casuarine 4. N-deprotection of 81 using catalytic hydrogenolysis provided pyrrolidine 82 which was subsequently transformed to 83 by refluxing in methanol in the presence of a catalytic amount of NaOMe. Acetylation under standard reaction conditions then produced the acetate derivative 84 in an 88% yield. Reduction of the lactam carbonyl group of 84 using BH$_3$·SMe$_2$ complex in THF gave 85 in 96% yield. O-TBDPS deprotection and then debenzylation provided 86 in 73% yield. Hydrogenolysis then gave the final compound, however it was not pure. The product was further purified by per-acetylation that gave 87 in 88% yield. Base catalysed deacetylation of 87 afforded 3-epi-casuarine 4 in 66% yield. This synthesis was achieved in 12 steps from the pyrrolidine derivative 76 in an overall yield of 2.2%.
Scheme 12. Total synthesis of 3-epi-casuarine 4 by Izquierdo et al. [45]. Reagents and conditions: (a) CbzCl, Me2CO, K2CO3, rt, 25%; (b) TPAP, NMO, 4°A Ms, CH2Cl2, 64%; (c) Ph3P=CHCO2Me, CH2Cl2, rt, 93%; (d) OsO4, NMO, DHQ-CLB, acetone/H2O, rt, 2 d, (80:81 = 13%:84%); (e) H2, 10% Pd-C, MeOH; (f) cat. NaOMe, MeOH, rt, 63%; (g) Ac2O, py, DMAP, 88%; (h) BH3·SMe2, THF, then MeOH, Δ, 96%; (i) n-Bu4N+F-·3H2O, THF, rt, 73%; (j) i: H2, 10% Pd-C, MeOH, then Amberlite IRA-400 (OH⁻ form), ii: Ac2O, py, DMAP, 70%; (k) cat. NaOMe, MeOH, rt, 66%.
In 2006, Fleet et al. [6] reported the synthesis of casuarine 1 (Scheme 4) together with the synthesis of 3-epi-casuarine 4 from D-gluconolactone 27 in the same publication (Scheme 13). [6] He followed the same methodology that he used to synthesize casuarine 1 up to the precursor 34. Regioselective protection of the primary hydroxyl group of diol 34 with TBSCI and then reaction at the secondary hydroxyl group by treatment with methanesulfonyl chloride generated the mesylate 88 in 66% yield. Reduction of the lactam carbonyl group of 88 with BH₃·THF gave the protected amine 89 (57% yield). Finally pure 3-epi-casuarine 4 was obtained after 2 more steps; (i) O-silyl group hydrolysis with TFA to produce 90; and then (ii) cyclization by treatment with sodium acetate (89% yield over the two steps).

Ritthiwigrom and Pyne’s synthesis of 3-epi-casuarine 4 [31] started with the precursor 56 which was prepared in 9 steps from L-xylose (Scheme 14). Their syntheses required an inversion of the
configuration of the butyl side-chain secondary hydroxyl group in 56. This was achieved by the Mitsunobu reaction of 56 using 4-nitrobenzoic acid [46]. Base treatment (K2CO3/MeOH, rt, 1 d) of the resulting secondary 4-nitrobenzoate ester resulted in benzoate hydrolysis. Cyclization under Mitsunobu reaction conditions, using toluene rather than pyridine as the solvent, gave a separable mixture the desired pyrrolizidine 92 in 70% yield and the indolizidine product 93 in 4% yield. The epoxy-pyrrolizidine 92 was treated with NaHSO4/CH2Cl2 under the conditions that were described in Scheme 7 except that heating at 50 °C was continued for 7 days (Scheme 14). The slower rate of epoxide ring-opening of 92, when compared to that of 3-epi-92, was attributed to the increased steric hindrance of the α-face of the epoxide moiety due to the 3-α-CH2OTBS substituent. Because of the slow reaction rate hydrolytic cleavage of the OTBS group also occurred resulting in a mixture of products that was difficult to separate. Acetylation of the mixture and then separation by column chromatography gave the desired acetylated product 94 (7% yield), the epoxide 96 in 9% yield and the undesired tricyclic bridged ether products 95 (8%) and 97 (17%) (Scheme 14). The isolation of epoxide 96 indicated that OTBS cleavage was faster than either the intermolecular or intramolecular (leading to tricyclic bridged ether products) epoxide ring-opening reactions. Unfortunately, several attempts to improve the yield of the desired product 94 were not successful. While the use of a more stable protecting group for the primary alcohol group in 92 may have been more efficient, this variation was not examined. Hydrogenolysis of 94 over PdCl2/H2 in MeOH for 4
days gave diastereomerically pure 3-epi-casuarine 4 in 77% yield after purification by ion-exchange chromatography.

Scheme 14. Total synthesis of 3-epi-casuarine 4. [31] Reagents and conditions: (a) i: p-NO2ArCO2H, PPh3, toluene, 80 °C, 5 h; ii: K2CO3, MeOH, rt, 24 h, 50%; (b) DIAD, Ph3P, toluene, 80 °C, 3 d; (c) i: NaHSO4, CH2Cl2, 50 °C, 7 d; ii: Ac2O, py, DMAP, 24 h; (d) PdCl2, H2 (1 atm), MeOH, 4 d; ion-exchange, 77%.
CONCLUSIONS

In summary, we have described the isolation, structure elucidation, glycosidase inhibitory activities and the synthesis of the four naturally occurring casuarines, namely, casuarine 1, casuarine-6-\(O\-\alpha\)-D-glucoside 2, 6-epi-casuarine 3 (uniflorine A) and 3-epi-casuarine 4. Casuarine 1 is a potent inhibitor of \(\alpha\)-D-glucosidases whereas casuarine-\(O\-\alpha\)-glucoside 2 was found to be a more active inhibitor of \(\beta\)-D-glucosidase. Both compounds 1 and 2 were potent inhibitors of amyloglucosidase from \textit{Aspergillus niger}. Casuarine 1 was found to be a potent inhibitor of the human \(N\)-terminal subunit of maltase-glucoamylase (NtMGAM) which could potentially aid in the treatment of type-II diabetes. Casuarine-\(O\-\alpha\)-glucoside 2 was found to be a potent (nM) trehalase inhibitor with potential use as an insecticide. X-ray structural analysis and computer aided docking studies of these natural products bound to these target enzymes have been carried out. These studies may assist in the design of more potent inhibitors in future studies. The originally assigned structures of uniflorine A and B were found to be incorrect. Based on careful NMR analysis and the total synthesis of uniflorine A, their structures were concluded to be 6-epi-casuarine 3 and casuarine 1, respectively. 6-Epi-casuarine 3 was found to be an inhibitor of \(\alpha\)-glucosidases and amyloglucosidase from \textit{Aspergillus niger}. In contrast to casuarine 1, 3-epi-casuarine 4 showed weak activity against three \(\alpha\)-D-glucosidases (from yeast, rice and \textit{Bacillus}) and was more active against \(\beta\)-D-glucosidase from almond. Future phytochemical studies may unveil other new casuarine epimers or glycoside derivative natural products.
ABBREVIATIONS

[α] = specific rotation
Ac = acetyl
Boc = tert-butoxycarbonyl
Bn = benzyl
Bz = benzoyl
Cbz = benzylxycarbonyl
DH = dihydroxylation
DHQ-CLB = O-(4-chlorobenzoyl)hydroquinine
DIBAL = di-iso-butylaluminium hydride
DMAP = 4-(N,N-dimethylamino)pyridine
DMDP = 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine
DMF = dimethylformamide
Fmoc = 9-fluorenylmethoxycarbonyl
IC50 = 50% inhibitory concentration
Ki = the dissociation constant for binding of inhibitor to enzyme
TDS = 1,1,2-trimethylpropylsilyl
THF = tetrahydrofuran
μM = micro molar
NMO = N-methylmorpholine N-oxide
NOE = NOE nuclear Overhauser effect
nM = nano molar
NtMGAM = N-terminal subunit of maltase-glucoamylase
RCM = ring-closing metathesis
ROESY = rotating frame Overhauser effect spectroscopy
TBAF = tetrabutylammonium fluoride
TBDPS = tert-butyldiphenylsilyl
TBS = tert-butyldimethylsilyl
TMS = trimethylsilyl
TPAP = tetrapropylammonium perruthenate
Tre37A = Escherichia coli trehalose

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